Asprosin aggravates nonalcoholic fatty liver disease via inflammation and lipid metabolic disturbance mediated by ROS

Chaowen Wang
Nanchang University

Wenjing Zeng
Nanchang University

Li Wang
Nanchang University

Xiaowei Xiong
Nanchang University

Shen Chen
Nanchang University

Qianqian Huang
Nanchang University

Guohua Zeng
Nanchang University

Qiren Huang ( qrhuang@ncu.edu.cn )
Nanchang University

Research Article

Keywords: Asprosin, Fat deposition, Nonalcoholic fatty liver disease, Nonalcoholic steatohepatitis

Posted Date: September 14th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3323240/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

Asprosin (ASP) is a newly-identified adipokine and plays important roles in energy metabolism homeostasis. However, there is no report on whether and how ASP is involved in the pathogenesis of nonalcoholic fatty liver disease (NAFLD). Therefore, in this study, we investigated the role and the underlying mechanisms of ASP in the cell and mouse models of NAFLD. Our data showed that ASP-deficiency significantly alleviated HFD-induced inflammation and NAFLD, inhibited the hepatic fat deposition and down-regulated the expressions of fat acid synthase (FASN), peroxisome proliferator-activated receptor γ (PPARγ) and forkhead box protein O1 (FOXO1) in the ASP-deficiency mouse model, suggesting that ASP is involved in the pathogenesis of NAFLD. Moreover, we found that the mechanism of ASP responsible for NAFLD was through disturbing the lipid metabolism homeostasis of hepatocytes and promoting the inflammation mediated by ROS. The findings suggest that ASP would become a diagnostic marker and therapeutic target for NAFLD.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of most the prevalent chronic disease around the world, characterized as excessive accumulation of liver fat, accounting for approximately 25% of the worldwide population [1]. With the development of NAFLD, its death risk is significantly higher than that in the healthy individuals at all stages [2]. In order to elucidate its pathogenesis, in 2020, an international consensus was reached which renames the NAFLD to the metabolism impairment associated with fatty liver [1, 3]. Currently, NAFLD is thought as a complicated metabolic disorder caused by the intricate interactions among genetic susceptibility, host metabolic characteristics and the environmental factors [4]. Among the interactions, the crosstalk between the lipid metabolic disorders and the inflammatory responses is supposed to be the most important, which jointly promote the initiation and progression of NAFLD.

Growing study has demonstrated that lipid enrichment is a key for NAFLD [5]. The accumulation of excess fatty acids in liver cells will increase the production of lipotoxic substances, which in turn cause oxidative stress and promotes the free of pro-inflammatory cytokines [6]. Hepatic steatosis is triggered by an imbalance between lipid enrichment and clearance, and the main regulatory pathways involved include free fatty acid uptake, lipogenesis and fatty acid-oxidation so on [7]. The lipid metabolism pathways are regulated by various factors and the disruption of the energy metabolism pathways results in hepatic lipid deposition, which in turn leads to NAFLD [8, 9].

Adipokines are a class of bio-active peptides secreted by adipocytes. They play important functions in the maintenance of energy dynamic equilibrium, inflammation and immune response, inflammation and immune response [10]. Asprosin (ASP) is a fasting-induced glycogenic hormone that discovered and first described in 2016 [11, 12]. Many research have proved that ASP plays a key role in promoting the release of glucose from the liver and maintaining glucose homeostasis [13, 14]. A decrease in the circulating ASP level prevents the hyperinsulinemia-associated with metabolic syndrome. Abnormally increased ASP
serum concentrations are seen in people with type 2 diabetes and obesity [15, 16]. It is reported that the serum levels of ASP are also abnormally elevated in the children with NAFLD and obesity [17]. At present, there is no such a treatment as targeting at ASP in the liver for NAFLD. Therefore, this study investigated the liver fatty lesions induced by high-fat diet (HFD) in the ASP-deficient (ASP<sup>−/−</sup>) mouse model to evaluate the relationship between ASP and the progression of NAFLD. Moreover, LO2 cells were used to explore the possible mechanism of ASP on NAFLD. These findings would provide a new target for the prevention and treatment of NAFLD, and deliver the basis for the research and development of novel drugs targeted at ASP.

2. Materials and methods

2.1 Animal experiment protocol

The animal experiments were granted by the Laboratory Animal Welfare and Ethic Committee of Nanchang University And conducted in comply with the Guidelines for the Protection and Use of Laboratory Animals issued by the National Institutes of Health (NIH Publication No. 85 – 23, revised in 1996). Twelve male wild-type (WT) C57BL/6 mice (SPF grade, Laboratory Animal Science Center of Nanchang University) were randomly allocated into WT-ND group and WT-HFD group. At the same time, 12 male adipose tissue specific ASP-deficient (ASP<sup>−/−</sup>) C57BL/6 mice (SPF grade, Shanghai Model Organ Center, Shanghai, CHN) were divided into ASP<sup>−/−</sup>-ND and ASP<sup>−/−</sup>-HFD group, and fed with normal diet (ND) and high-fat diet (HFD) for 16 weeks, respectively, with 6 mice in each group. Body weights were measured every 4 weeks, and finally the mice were euthanized with 3% (v/v) isoflurane and sacrificed by cervical dislocation. Liver was used to calculate the liver index (LI = liver wet weight (g)/body weight (g) ×100%).

2.2 Measurement of biochemical parameters in serum and liver tissue

Indicators in mice serum and liver tissue were tested according to manufacturer's instructions, include triglycerides (TG, A110-1-1) and total cholesterol (TC, A111-2-1), alanine aminotransferase (ALT, C009-2-1), aspartate aminotransferase (AST, C010-2-1). All kits were purchased from Nanjing Jiancheng Bioengineering Institute, CHN.

2.3 Histological analysis

Liver tissue was embedded in tissue OCT complex. Frozen sections were prepared and sectioned into 6 μm thick sections and stained with oil red O dye to evaluate the lipid accumulation degree, stained with hematoxylin-eosin (H&E) staining to assess the liver injury. The sections were observed and captured with a camera equipped with a light microscope (Nikon, JPN). All kits were purchased from Solarbio (Beijing, CHN).

2.4 Immunofluorescence staining
Frozen sections were prepared. For immunofluorescence chemical detection, the sections were first hatched overnight at 4°C with a primary antibody against murine CD11b (WL1193, Wanleibio, CHN). Then, the sections were stained with fluorophore-labeled secondary antibodies (HA1016). Nuclei were stained with DAPI (Solarbio, Beijing, China). The immunofluorescence images were captured with a camera equipped with a fluorescence microscope (Nikon, Tokyo, Japan).

2.5 Cell culture and treatment

LO2 cells line (Collection of the Chinese Academy of Sciences) cultured in RPMI 1640 medium (G4531, Servicebio, Wuhan, CHN) added with 10% fetal bovine serum (TBD11HT, Haoyangbio, Tianjin, CHN) and in a dank environment containing 5% CO2 at 37°C. The LO2 cells were treated with 0.2 mM free fatty acid (FFA) formula (oleic acid: palmitoleic acid = 2:1 preparation) to establish a lipid accumulation model [18]. The LO2 cells were randomly allocated into control (ctrl), FFA, ASP and ASP + FFA groups. The cells in ASP and ASP + FFA groups were treated with 50 µM ASP for 48 h in the absence or presence of 0.2 mM FFA and the cells in control group were not treated with FFA and ASP. Human recombinant ASP was bought from China Peptides Biotech, Inc. (Suzhou, CHN).

2.6 Enzyme-linked immunosorbent assay (ELISA)

The levels of IL-6 and tumor necrosis factor-α (TNF-α) from mouse serum or cell media were detected with the commercial ELISA kits (TNF-α,2H-KMLJh311776; IL-6, 2H-KMLJ31201mm) which were purchased from Camilo (Nanjing, CHN). The detection was in compliance with the manufacturer’s instructions.

2.7 RNA extraction and real-time quantitative PCR

The LO2 cells were homogenized with TRIzol reagent (Invitrogen, Waltham, MA, USA) and total RNA was extracted. Subsequently, perform RNA reverse transcription (Reaction conditions: 37 °C, 15 min;85 °C, 5 s) and qRT-PCR (Reaction conditions: 94°C, 30 s; 94 °C, 5 s; 55 °C, 15 s; 72 °C, 10 s (42 cycles)) according to the manufacturer’s instructions. All kits were purchased from Solarbio (Beijing, CHN). The primer sequences are as follows: acyl-CoA oxidase 1 (ACOX1 GenBank registration number: NM_001278352.2; 122 bp), forward, 5'-CTCACTCGAAGCCAGCGTTA − 3', reverse, 5'-CGGTGCACAGAGTTTTAAACCA-3'; lipoprotein lipase (LPL GenBank registration number: NM_000237.3; 137 bp), forward, 5'-CCAGATTGTTGCAGCGGTTC-3', and reverse, 5'-CGAGCGCTCCATTCCATCTCT-3'; β-actin (GenBank registration number: NM_001101.5; 81BP), forward, 5'-GGGCATGGGTCAGAAGATT-3', and reverse, 5'-TCGATGGGGTGACTCCAGGGT-3'. All primers were synthesized by Generay Biotechnology (Shanghai, CHN).

2.8 Western blotting

Liver tissues or LO2 cells were lysed with RIPA lysis buffer containing protease inhibitors (Pulley, Beijing, CHN), and the protein concentrations were determined with the BCA Protein Analysis Kit (Tiangen, Beijing, CHN). The protein samples (30 µg) were electrophorized and transferred to PVDF membrane (Millicon, Burlington, Massachusetts, USA). The PVDF membrane was blocked with 7% (w/v) nonfat milk powder.
for 2 h, and then incubated with the corresponding primary antibody solution (1:1000) at 4°C overnight, followed by an incubation in a secondary antibody solution (1:2000) for 2 h. Subsequently, the membrane was developed by electrochemiluminescence (ECL) detection kit (Bayside, Shanghai, CHN) and finally the bands were observed and analyzed with a Gel Imaging System (Bio-Rad Lab, Hercules, USA). The expression levels were expressed with the grayscale value by Image J software (NIH, Bethesda, MD, USA). The commercial primary antibodies used in this study were as follows: anti-NF-κBp65, anti-p-NF-κBp65 and anti-PPARγ (Proteintech Biotech, Inc., Wuhan, CHN), anti-Acetyl-CoA Carboxylase (ACC, Thermo, Shanghai, CHN), anti-IKKα/β and anti-p-IKKα/β (Santa Cruz Biotechnology Co., Ltd. Beijing, CHN), anti-IκBα (Abmart Biotech, Inc., Shanghai, CHN), anti-FASN and anti-FOXO1 (Abcam, CA, USA) and anti-β-actin (Fude Biotech, Inc., Hangzhou, CHN).

2.9 Statistical analysis

All data were indicated as mean ± SD and statistically analyzed using GraphPad Prism 8.0. The homogeneity of variance and one-way or two-way ANOVA were performed, followed by an unpaired Student’s t-test, if applicable. The difference was considered significant if p < 0.05.

3. Results

3.1 ASP-deficiency alleviates HFD-induced lipid metabolic disturbance in mice

To study the effects of ASP on NAFLD, we constructed the adipose tissue ASP-deficiency mice which was described in our recently-published paper [19]. As we previously reported, after the specific deletion of ASP in the adipose tissue, the ASP levels both in the adipose tissue and the circulating ASP decreased significantly [19]. Although it has shown that the skin and pancreas β cells may also secrete ASP [18], our results showed that compared to the WT-ND mice, the mice in the WT-HFD group exhibited the phenotype of NAFLD with obesity, presenting that the body weight (Fig. 1A), liver weight (Fig. 1B) and the ratio of liver wet weight (g) to body weight (g) (Fig. 1C) increased significantly; besides, serum triglycerides (TG) (Fig. 1D) and total cholesterol (TC) (Fig. 1E) as well as hepatic tissue TG (Fig. 1F) and TC (Fig. 1G) markedly elevated. Although there was no significant change in these parameters between the WT-ND and ASP−/−-ND mice, the above-mentioned parameters significantly reduced in the ASP−/−-HFD group, compared to the WT-HFD group, suggesting that the ASP-deficiency attenuates the HFD-induced lipid metabolic disturbance in mice.

3.2 ASP-deficiency mitigates the liver fat deposits and steatosis induced by HFD in mice

The deposition of liver fat is a trigger of the hepatic steatosis. Therefore, we next evaluated the effects of ASP on the fat deposition in the liver of mice. The results of the oil red O staining displayed that the number and size of lipid droplets in the liver of the WT-HFD group mice significantly increased compared
with those of the WT-ND group mice. However, the ASP-deficiency markedly mitigated the liver fat deposits and steatosis induced by HFD (Fig. 2A, B). As important modulators of lipid anabolism, the levels of FASN, FOXO1 and PPARγ were detected in the liver tissues. The western blot analysis results showed that the levels of FASN, FOXO1 and PPARγ in the WT-HFD group mice were respectively elevated by approximately 1.2, 1.2 and 1.3 fold of WT-ND, whereas the ASP-deficiency normalized the increments induced by HFD (Fig. 2C-E). Taken together, these data demonstrate that the ASP-deficiency mitigates the liver fat deposits and steatosis induced by HFD likely via the down-regulation of FASN, FOXO1 and PPARγ.

3.3 ASP-deficiency alleviates mouse nonalcoholic steatohepatitis

The hepatic steatosis would transform to nonalcoholic steatohepatitis (NASH) if it were out of the effective control. Therefore, we then evaluated the effects of ASP on NASH in mice. As anticipated, the mice in the WT-HFD group manifested as a NASH phenotype, evidenced by considerable elevations in serum AST and ALT whereas the ASP-deficiency reversed the elevations induced by HFD (Fig. 3A, B). As there exists infiltration of a large number of monocytes and neutrophils in NASH, we subsequently investigated the effects of ASP on the infiltration using a specific antibody against CD11b which is a marker of monocytes and neutrophils. The immunofluorescence (IF) results revealed that compared with the WT-ND mice, the WT-HFD group mice had more inflammatory cell infiltration into liver tissue; compared with the WT-HFD group, the inflammatory infiltration of liver tissue in the ASP−/−-HFD group mice was significantly reduced (Fig. 3C, D). Moreover, the results from the H&E staining of liver tissue were consistent with the immunofluorescence results (Fig. 3E). Besides, we observed the effects of ASP on the inflammation pathway IKKa/β-NF-κBp65. The western blot analysis results unveiled that the IKKa/β-NF-κBp65 pathway in the WT-HFD group mice were activated, whereas the ASP-deficiency significantly blocked this pathway activation induced by HFD (Fig. 3F-H).

3.4 ASP treatment aggravates dysfunction and lipid aggregation induced by FFA in LO2 cells

Subsequently, the effects of ASP on hepatic dysfunction and lipid accumulation were evaluated at the cellular levels. LO2 cells, a normal human hepatocyte line, were treated with either FFA, ASP alone or ASP combination with FFA. The results showed that like FFA, the ASP treatment alone significantly elevated the levels of AST and ALT compared to the control (ctrl) group; moreover, the ASP + FFA treatment displayed more potent effects than the ASP treatment alone (Fig. 4A, B), demonstrating that ASP treatment aggravates the hepatic dysfunction induced by FFA. In addition, the ASP treatment (ASP) alone did not affect but worsened the elevation of the TG levels induced by FFA (ASP + FFA) (Fig. 4C); however, neither ASP nor ASP + FFA had significant effects on the TC levels (Fig. 4D). Oil red O results showed that similar to FFA, the ASP treatment alone significantly promoted the lipid accumulation; moreover, the ASP
+ FFA treatment had a more potent effect than the ASP treatment alone, suggesting that ASP reinforces the lipid aggregation induced by FFA in LO2 cells (Fig. 4E, F).

3.5 ASP treatment disturbs the lipid metabolism homeostasis in LO2 cells

The lipid accumulation results from the imbalance of the lipid metabolism homeostasis, especially anabolism overtaking catabolism. Thus, we investigated the effects of ASP treatment on the expressions of the markers responsible for the lipid anabolism and catabolism. The western blot analysis results showed that like FFA, both ASP treatment alone and ASP + FFA treatment significantly increased the expression levels of FASN, PPARγ, FOXO1 and ACC which are responsible for the anabolism (Fig. 5A-E) whereas decreased the mRNA levels of ACOX1 and LPL which are responsible for the catabolism (Fig. 5F, G), which is consistent with the results in vivo. Collectively, our data demonstrates that ASP treatment disturbs the lipid metabolism homeostasis through promoting the anabolism and inhibiting the catabolism of lipid in vitro.

3.6 ASP treatment exacerbates the inflammation induced by FFA in LO2 cells

We have demonstrated in the in vivo experiments that the ASP-deficiency effectively alleviates the inflammation. Finally, we investigated whether the ASP treatment would accelerate inflammation in LO2 cells. Our results showed that FFA (0.2 mM) significantly increased the levels of IL-6 and tumor necrosis factor- (TNF- ) compared with the control group. Like FFA, ASP alone significantly also increased the levels of IL-6 and TNF-. Moreover, the combination treatment of ASP and FFA exhibited a synergistic effect (Fig. 6A, B). Besides, the western blot results showed that Like FFA, ASP alone significantly elevated the levels of P-IKKα/β and P-NF-κBp65 but decreased the IκB levels; moreover, the combination treatment of ASP and FFA also exhibited a synergistic effect, suggesting that ASP can exacerbate the inflammation induced by FFA (Fig. 6C-F). It is well-known that the oxidative stress mediated by ROS is a primary inducer for the lipotoxicity and inflammation. Therefore, we examined whether ASP would exert the effects on the ROS levels. Unexpectedly, unlike FFA, although the ASP treatment alone did not significantly increase the ROS levels, it substantially elevated the ROS levels in the presence of FFA (Fig. 6G, H), indicating that ASP exacerbates the inflammation via the oxidative stress mediated by ROS.

4. Discussion

NAFLD is collectively referred as a cluster of progressive liver diseases characterized by excessive accumulation of TG and cholesterol in liver, ranging from simple hepatic steatosis, NASH, hepatic fibrosis to cirrhosis and even hepatocellular carcinoma [20]. Unfortunately, except diet control and physical exercise, there are no potent drugs for NAFLD to date. Therefore, it is urgent to research and develop effective pharmacological treatments to combat NAFLD [21]. In the study, we found that ASP is involved
in the occurrence and development of hepatic steatosis and NASH and the ASP-deficiency alleviates the liver fat deposits and steatosis as well as NASH in mice, indicating that the negative intervention targeted at ASP would be an effective therapeutic measure for NASH.

ASP is an adipokine mainly secreted by white adipose tissue. It plays extensive patho-physiological roles in glyco-lipid metabolism and appetite control etc [22]. Our recent studies found that ASP causes and even exacerbates the vascular endothelium dysfunction through promoting the mitochondrial fission in obesity model [19]; Moreover, ASP exerts the pro-inflammation effect through activating the IKKβ-NF-κBp65 pathway other than the oxidative stress pathway in vascular endothelial cells [23]. In this study, we found that ASP exacerbates the inflammation via the oxidative stress mediated by ROS in L02 cells. There is a conflict between our present studies and previous studies. In our opinion, the cause of the conflict might be different in the cells used.

To verify the effects of ASP on NAFLD in mice, we established the ASP-deficient mice by knockouting the exon 65 and 66 in profibrillin gene (Fbn1). The ASP-deficient mice exhibits the phenotypes including decreased circulating ASP levels and a lean appearance [19]. Our present results also show that the ASP-deficiency can effectively decrease the body weight and liver weight of mice, which is consistent with the phenomenon that the individuals with obesity are more prone to suffering from NAFLD [24]. It is well-known that liver is one of the main target organs of ASP. ASP promotes the hepatic glucose release in the fasting state via a putative olfactory receptor (OLFR734) dependent cAMP-PKA pathway [25, 26]. However, little is known whether and how ASP involves in the occurrence and progression of NASH. In our study, we found that the ASP-deficiency mitigates the liver fat deposits, steatosis, and NASH induced by HFD in mice.

In early stage of NAFLD, the accumulation of lipid especially TG in liver is a trigger. The lipid metabolic disturbance especially anabolism exceeding catabolism results in the accumulation of lipid. PPARγ, FOXO1, FASN and ACC are the critical factors which prompts the lipid anabolism [20, 27], whereas ACOX1 and LPL are the crucial factors responsible for the lipid catabolism [28]. It was documented that over-expression of FOXO1 in the liver contributes to an increase in TG synthesis and a decrease in fatty acid oxidation, thereby exacerbating the hepatic steatosis, and the negative effects of FOXO1 on NAFLD have also been demonstrated [29]. Like FOXO1, PPARγ is a key transcription factor which is involved in the lipogenesis. FASN converts dietary sugars to fatty acids, for example, palmitate, which plays a major role in the liver's production of excess fat and certain lipotoxic molecules. ACC, a rate-limiting enzyme of the fatty acid synthesis, plays an important role in the biosynthesis of fatty acids [30]. Conversely, ACOX1, a rate-limiting enzyme of fatty acid β-oxidation, is involved in the catabolism of fatty acids [31]. LPL takes part in the degradation of lipoproteins in several biochemical process [28]. Our in vivo and in vitro results have shown that ASP promotes the lipid accumulation in liver and hepatocytes mainly through up-regulating the anabolic factors and down-regulating the catabolic factors.

Inflammation is a marker and driver of nonalcoholic steatohepatitis progression. The inflammatory response of hepatocytes is thought to be primarily triggered by the lipotoxic effects of TG [29]. TG
induces the generation of ROS and endoplasmic reticulum stress, thus promoting the release of pro-inflammatory cytokines from hepatocytes through activating the IKK β-NF-κBp65 pathway [32]. Subsequently, inflammatory cells are activated and attracted to the liver, releasing cytokines and further exacerbating the liver's inflammatory response. Importantly, we also found that ASP causes a sharp increase in ROS, which leads to oxidative stress and exacerbates the occurrence of inflammation. ASP also promotes TG aggregation under the eutrophication, leading to such a predisposition to be susceptible to inflammation, and presenting the direct adverse effects of ASP on the liver. Our results show that the ASP-deficiency effectively inhibits the NF-κBp65 pathway in the NAFLD mice induced by HFD. Moreover, NF-κBp65 downstream inflammatory gene expressions are down-regulated and inflammatory cell infiltration is reduced. Similar results were obtained from the in vitro experiments. Besides its direct activating effects on the IKK β-NF-κBp65 pathway, ASP promotes the release of inflammatory factors such as IL-6 and TNF-α by LO2 cells, aggravating the occurrence of inflammation.

Of course, there are some shortcomings in the study. It is well-known that the diagnostic gold standard for NAFLD is the biopsy, an invasive test that can cause irreversible damage [17]. However, our in vivo experimental model was only evaluated through phenotypic analysis and some biochemical parameters, and there are some deviations. Also, as a secreted adipokine, it is unclear what are the specific targets of ASP on the membrane of hepatocytes. Furthermore, it is still uncertain how the exact crosstalk between the inflammatory pathway and lipid metabolic pathway mediated by ASP promotes the pathogenesis of NAFLD. All these issues need to be further investigated in the future.

5. Conclusions

In summary, this study provides solid evidence for the association of ASP with NAFLD. Our in vitro results have demonstrated that ASP treatment aggravates hepatocyte dysfunction and lipid aggregation, disturbs the lipid metabolism homeostasis, exacerbates the inflammation via the oxidative stress mediated by ROS. More importantly, the ASP-deficiency by gene deletion alleviates the liver fat deposits and steatosis as well as NASH in mice (Fig. 7), indicating that the negative intervention targeted at ASP would be a promising therapy approach for NASH. Besides, the circulating ASP levels might be regarded as a good diagnosis marker for the obesity accompanied by NAFLD in the future.

Declarations

Funding

This study was supported by the grants from the National Natural Scientific Foundation of China (819600153 and 82260726) and Jiangxi Provincial Natural Science Foundation (20232ACB206061).

Declaration of competing interest

None
CRediT authorship contribution statement

Chaowen Wang: Conceptualization, Formal analysis, Investigation, Data curation, Writing-original draft, Writing-review & editing. Wenjing Zeng: Investigation, Data curation. Li Wang: Formal analysis, Data curation. Xiaowei Xiong: Conceptualization. Sheng Chen: Formal analysis. Qianqian Huang: Data curation. Guohua Zeng: Funding acquisition. Qiren Huang: Conceptualization, Writing-review & editing, Supervision, Funding acquisition. All authors have read and agreed to the published version for the manuscript.

Data availability

All the data supporting the findings of the study are shown in this paper and are available upon reasonable request.

Ethics approval

All animal experiments were approved by the Laboratory Animal Welfare and Ethic Committee of Nanchang University (no. 2021-0019) and conducted in accordance with the guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996).

Consent to publish

The Author confirms: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, review, or thesis); that it is not under consideration for publication elsewhere; that its publication has been approved by all co-authors, if any; that its publication has been approved (tacitly or explicitly) by the responsible authorities at the institution where the work is carried out.

References


**Figures**
Figure 1

**ASP-deficiency alleviates HFD-induced lipid metabolic disturbance in mice**

Body weight (A), liver tissue weight (B) and liver organ index (C) were measured and calculated in C57BL/6 mice. Serum TG (D) and TC (E) as well as liver TG (F) and TC (G) were detected. Results were expressed as mean ± standard deviation (n = 6). One-way ANOVA was performed, followed by an LSD post-hoc test. *P < 0.05, **P < 0.01, ***P < 0.001, ns: no significance, vs. WT-ND group; #P < 0.05, ##P < 0.01, vs. WT-HFD group; $P < 0.05, $$$P < 0.01, vs. ASP^{-/-}-ND group. WT-ND: wild-type mice fed with normal diet, WT-HFD: wild-type mice fed with HFD, ASP^{-/-}-ND: ASP deficient mice fed with normal diet, ASP^{-/-}-HFD: ASP deficient mice fed with HFD. TG: triglyceride, TC: total cholesterol.
Figure 2

ASP-deficiency mitigates the liver fat deposits and steatosis induced by HFD in mice

Slides of mouse liver were stained with oil red O dye and visualized under a light microscope (A, scale bar = 50 μm). Oil red O dye was eluted with 100% isopropanol and the absorbance at 510 nm (A510) was determined using a microplate reader (B). Expressions of FASN (C and D), FOXO1 (C and E) and PPARγ (C and F) were evaluated by western blotting in mouse liver tissue. Results were expressed as mean ± standard deviation (n = 3). One-way ANOVA was performed, followed by an LSD post-hoc test. *P < 0.05, **P < 0.01, ns: no significance, vs. WT-ND group; #P < 0.05, ##P < 0.01, vs. WT-HFD group; $P < 0.05, $$$P < 0.01, ns: no significance, vs. ASP−/−-ND group. WT-ND: wild-type mice fed with normal diet, WT-HFD: wild-type mice fed with HFD, ASP−/−-ND: ASP deficient mice fed with normal diet, ASP−/−-HFD: ASP deficient mice fed with HFD. FASN: fat acid synthase, FOXO1: forkhead box protein O1, PPARγ: peroxisome proliferator-activated receptor γ.
Figure 3

**ASP-deficiency alleviates mouse nonalcoholic steatohepatitis**

Serum AST (A) and ALT (B) were detected in mice. The *in situ* expression of CD11b was measured with immunofluorescence and relative fluorescence intensity was analyzed with image J (C and D, scale bar = 50 μm, Red: CD11b, Blue: DAPI). Slides of mouse liver were stained with H & E and visualized under a light microscope (E, scale bar = 50 μm). Expressions of IKKa/β, P-IKKα/β (F and G) and NF-κBp65, P-NF-κBp65 (F and H) were evaluated by western blotting in mouse liver tissue. Results were expressed as mean ± standard deviation (n = 3). One-way ANOVA was performed, followed by an LSD post-hoc test. *P < 0.05, **P < 0.01, ***P < 0.001, ns: no significance, vs. WT-ND group; #P < 0.05, vs. WT-HFD group; $P < 0.05, $$$P < 0.01, vs. ASP−/−-ND group. WT-ND: wild-type mice fed with normal diet, WT-HFD: wild-type mice fed with HFD, ASP−/−-ND: ASP deficient mice fed with normal diet, ASP−/−-HFD: ASP deficient mice fed with HFD. AST: aspartate aminotransferase, ALT: alanine aminotransferase, IKKa/β: inhibitory kappa B kinase α/β, NF-κB: nuclear factor kappa B.
**Figure 4**

**ASP treatment aggravates dysfunction and lipid aggregation induced by FFA in LO2 cells**

The levels of AST (A), ALT (B), TG (C) and TC (D) were detected in the medium of LO2 cells. Slides of hepatocytes were stained with oil red O dye and visualized under a light microscope (E, scale bar = 50 μm). Oil red O dye in hepatocytes was eluted with 100% isopropanol and the absorbance at 510 nm (A510) was determined using a microplate reader (F). Results were expressed as mean ± standard deviation (n = 3). One-way ANOVA was performed, followed by an LSD post-hoc test. *P < 0.05, **P < 0.01, ***P < 0.001, ns: no significance, vs. ctrl group; #P < 0.05, ns: no significance, vs. FFA groups; $P < 0.05, $$$P < 0.01, vs. ASP group. ctrl: control group; FFA: 0.2 mM FFA group; ASP: 50 μM ASP group; ASP+FFA: 0.2 mM FFA plus 50 μM ASP group. AST: aspartate aminotransferase, ALT: alanine aminotransferase, TG: triglyceride, TC: total cholesterol.
Figure 5

**ASP treatment disturbs the lipid metabolism homeostasis in LO2 cells**

Expressions of FASN (A and B), ACC (A and C), FOXO1 (A and D) and PPARγ (A and E) were evaluated by western blotting in LO2 cells. The mRNA levels of LPL (F) and ACOX1 (G) were assayed with Real-time PCR. Results were expressed as mean ± standard deviation (n = 3). One-way ANOVA was performed, followed by an LSD post-hoc test. *P < 0.05, **P < 0.01, ***P < 0.001, ns: no significance, vs. ctrl group; #P < 0.05, ##P < 0.01, ###P < 0.001, vs. FFA group; $P < 0.05, ns: no significance, vs. ASP group. ctrl: control group; FFA: 0.2 mM FFA group; ASP: 50 μM ASP group; ASP+FFA: 0.2 mM FFA plus 50 μM ASP group. ACC: acetyl-CoA carboxylase, FASN: fat acid synthase, FOXO1: forkhead box protein 01, PPARγ: peroxisome proliferator-activated receptor γ, ACOX1: acyl-CoA oxidase 1, LPL: lipoprotein lipase.
Figure 6

**ASP treatment exacerbates the inflammation induced by FFA in LO2 cells**

Levels of IL-6 (A) and TNF-α (B) were determined by ELISA in the medium of LO2 cells. Expressions of IKKα/β, P-IKKα/β (C and D), NF-κBp65, P-NF-κBp65 (C and E) and IκBα (C and F) were evaluated by western blotting in LO2 cells. ROS levels were detected and analyzed by flow cytometry (G and H). Results were expressed as mean ± standard deviation (n = 3). One-way ANOVA was performed, followed by an LSD post-hoc test. *P < 0.05, **P < 0.01, ***P < 0.001, ns: no significance, vs. ctrl group; #P < 0.05, ##P < 0.01, vs. FFA group; $P < 0.05, $$$P < 0.01, vs. ASP group. ctrl: control group; FFA: 0.2 mM FFA group; ASP: 50 μM ASP group; ASP+FFA: 0.2 mM FFA plus 50 μM ASP group. IL-6: interleukin 6, TNF-α: tumor necrosis factor α, IKKα/β: inhibitory kappa B kinase α/β, NF-κB: nuclear factor kappa B, IκBα: inhibitory Kappa B α, ROS: reactive oxygen species.
A putative work model for ASP

ASP secreted by white adipose tissue acts on the liver, leading to increased expressions of ACC, FASN, FOXO1 and PPARγ as well as decreased expressions of ACOX1 and LPL etc. Subsequently, the accumulation of TG occurs and lipotoxicity arises. Over-generation of ROS finally causes the injury of liver via oxidative stress.